

5 We have generated a panel of anti-human gc chain mAbs by immunizing and
screening as described above. Binding of purified mAbs to an L cell transfectant stably
expressing human gc chain, but not to the L cell parent was detected by standard
immunofluorescent staining, is shown in Figure 2. These data demonstrate their specificity
for human gc chain. These mAbs also recognize gc chain expressed on normal human cells
10 as shown by their binding to PHA-activated PBMC (Fig. 3).

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RNA was isolated from the anti-human gc chain hybridoma line (CP.B8) and used for first strand cDNA synthesis. Two micrograms of RNA was converted to cDNA using oligo dT (GibcoBRL Superscript preamplification system). A fraction of the cDNA reaction was used as a template for amplification in a reaction using 6 μ M of the light chain variable region primers ACE-149 [5' d(CTGGATATCGTAATGACCCAGTCTCCA):SEQ ID NO: 9] and ACE-150 [5' d(GTTAGATCTCCAGCTTGGTCCC): SEQ ID NO: 10] or the heavy chain variable region primers VH-01 [5' d(AGGTSMARCTGCAGSAGTCWGG):SEQ ID NO: 11] where S=C/G, M=A/C, R=A/G and W=A/T and VH-02 [5' d(TGAGGAGACGGTGACCGTGGTCCCTTGGCCCC):SEQ ID NO: 12], 0.2 mM dNTPs, 10% DMSO (light chain only), 20 mM Tris-Cl (pH 8.8), 10 mM KCl, 10 mM (NH₄)₂SO₄, 2 mM MgSO₄, 1.5 mM MgCl₂, 0.1% Triton X-100, 100 μ g/ml BSA, and 1.25 units *Pfu* polymerase (Stratagene, La Jolla, CA) for 30 cycles at 94°C for 1 min., 50°C for 2 min. and 72°C for 2 min. The PCR fragments were isolated from a low melt gel and then cloned using the Pharmacia Sure Clone kit into pNN05. The DNA sequence was determined and compared to the Genbank database where matches to heavy and light variable regions were found. In order to prove specificity of these sequences for the human gc chain, a signal sequence and constant region were cloned adjacent to the